



MODELLING

INTRODUCTION

How long can a patch last on an astronaut?

How much radioactive peptide will diffuse into the astronaut's body?

When should the astronaut pop the extra media packet?

These were the types of questions we were able to answer by using a combination of lab experiments and mathematical models. These computational models were used to evaluate how our patch works and how realistic our patch is so that one day, we can make our project a reality.

GROWTH CURVES

Our first step was to determine the lifetime of our bacteria, *Bacillus subtilis*, as it would determine the nature of how our peptide would be produced and the lifetime of our patch. This was done through experimental growth curves:

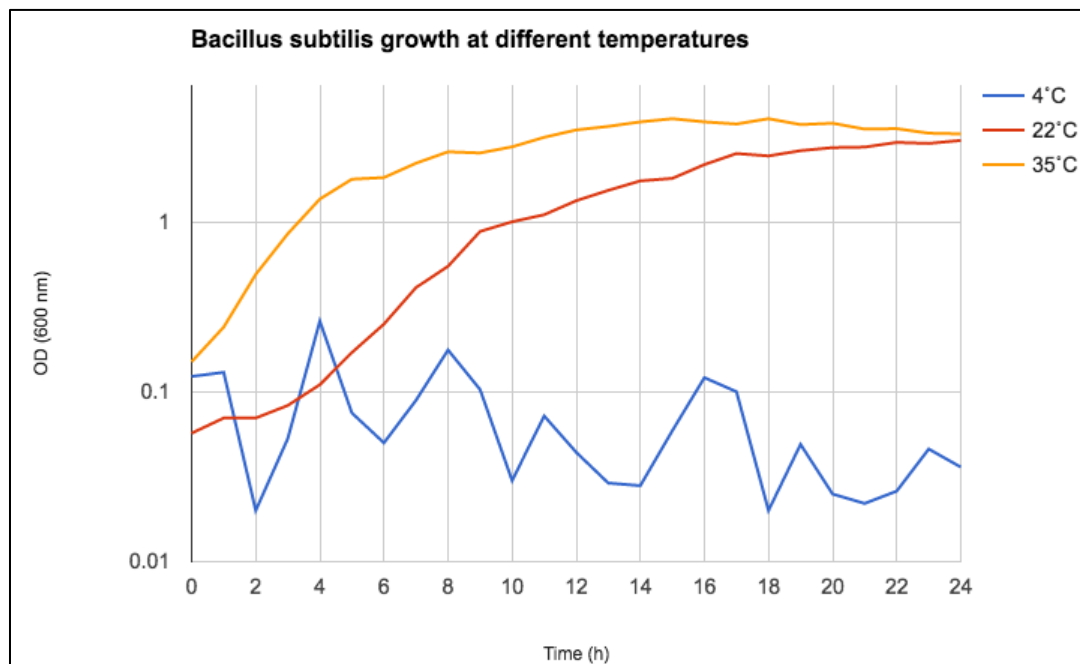


Figure 1: Raw data of growth curves at different temperatures. Three replicates of growth at various temperatures was measured every hour over a period of 24 hours. Optical density was measured by spectrophotometry at a wavelength of 600 nm (OD 600). The most relevant growth curve is the one in orange, as this is the average temperature of the surface of the skin.

The growth curves revealed that *B. subtilis*, under expected conditions, underwent death phase after 16-18 hours. This established when the packets of super-rich media in our patch

needed to be added to increase the bacteria's lifetime. For ease of remembrance for the user, we decided that the packets should be popped every 12 hours.

To simulate our patch, another experiment was conducted in which a packet of super rich media was added to the bacteria every 12 hours. This was in triplicates as there are three packets in our patch. Through this, we determined that the bacteria's lifetime in our patch is 60 hours (about 2.5 days). Since *B. subtilis* begins to release toxic material that may pass into the skin during its death phase (i.e. after 60 hours), we determined that the patch must be disposed of at this time.

The complete experiments can be found at http://2016.igem.org/Team:UofC_Calgary/Experiments.

DIFFUSION MODEL

MATLAB was used to develop a diffusion model to numerically represent the diffusion of our peptide, Bowman-Birk protease inhibitor (BBI), from the patch, through the skin and into the blood. This diffusion model was developed with the hopes to answer questions, such as:

Does the peptide reach a constant concentration in the blood while the patch is on?

Literature values show that the minimum required amount of peptide needed for radioprotection is 10 μM . Does the concentration in the blood reach 10 μM or higher?

How long does it take for the concentration in the blood to reach zero after the patch has been removed?

The following is a visual representation of what the system looks like.

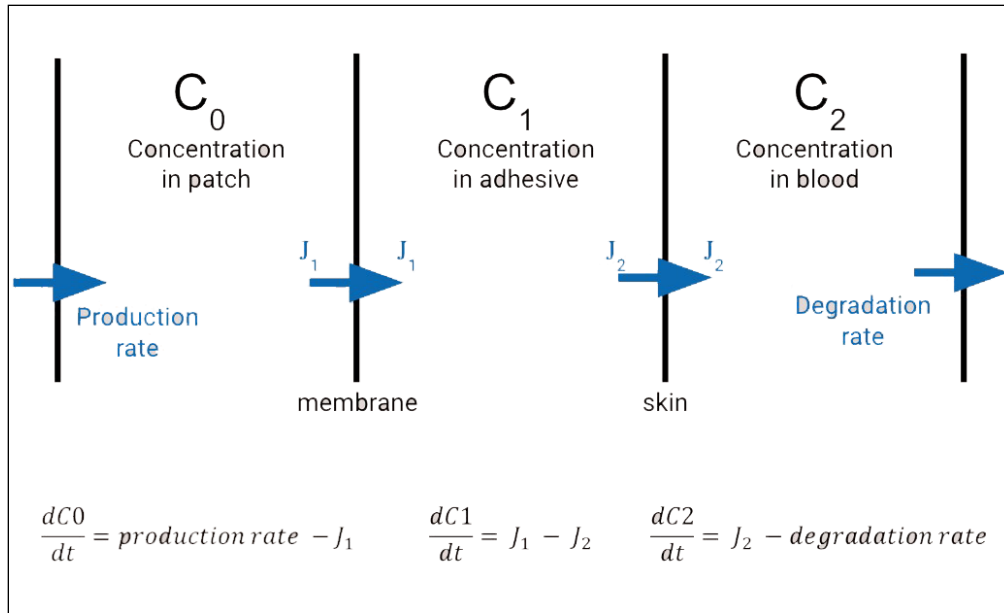


Figure 2: A visual representation of the diffusion model system. Note that the main barriers in this diffusion system are the patch's size-controlling membrane and the skin. The skin was assumed to be one homogeneous layer (other factors, such as differences in cellular structure of the skin, any absorption by cells and sweat ducts, enzymes, etc. were not considered).

To start developing our model, Fick's first and second law were used:

$$J = -D \frac{dC}{dx} \quad (1)$$

$$\frac{dC}{dt} = D \frac{d^2C}{dx^2} \quad (2)$$

Where:

J = concentration flux ($\frac{mol}{m^2 \cdot s}$)

D = diffusion coefficient ($\frac{m^2}{s}$)

C = concentration ($\frac{mol}{m^3}$)

x = distance (m)

t = time (s)

We were able to develop three equations (one for each section - see Figure 2) which were used to determine the concentration in the patch, C_0 , the concentration in the adhesive, C_1 , and the concentration in the blood, C_2 . These equations were developed on the basis that the change in concentration over time for a section is defined as the flux of peptide entering the section minus the flux of peptide leaving the section. To ensure that the units match, the flux variables were multiplied by the cross-sectional area and divided by the respective volume of the section:

$$\frac{dC_0}{dt} = \text{production rate} - J_1 \quad (3)$$

$$\frac{dC_0}{dt} = \text{production rate} - \left(\frac{\frac{-D_m \cdot (C_1 - C_0) \cdot x - \text{area}}{\Delta x_m}}{V_{\text{patch}}} \right) \quad (4)$$

$$\frac{dC_1}{dt} = J_1 - J_2 \quad (5)$$

$$\frac{dC_1}{dt} = \left(\frac{\frac{-D_m \cdot (C_1 - C_0) \cdot x - \text{area}}{\Delta x_m}}{V_{\text{adhesive}}} \right) - \left(\frac{\frac{-D_s \cdot (C_2 - C_1) \cdot x - \text{area}}{\Delta x_s}}{V_{\text{adhesive}}} \right) \quad (6)$$

$$\frac{dC_2}{dt} = J_2 - \text{degradation rate} \quad (7)$$

$$\frac{dC_2}{dt} = \left(\frac{\frac{-D_s \cdot (C_2 - C_1) \cdot x - \text{area}}{\Delta x_s}}{V_{\text{blood}}} \right) - \text{degradation rate} \quad (8)$$

Where the values are the following:

Table 1: Values Used In Equations (4), (6) and (8)

Parameter	Value	Where Value Is Obtained From
Cross sectional area, $x - \text{area}$ (m^2)	$4.9 \cdot 10^{-3}$	Patch is 7 cm by 7 cm
Diffusion coefficient of membrane, $D_m (\frac{m^2}{s})$	$2.88689 \cdot 10^{-10}$	[4] *Molecular weight (MW) in Equation 5 was used as 2579 g/mol. **The diffusion coefficient of the skin and the membrane were both assumed to be the same because they are both porous membranes.
Thickness of membrane, x_m (m)	$50.8 \cdot 10^{-6}$	3M information sheet
Volume of patch, V_{patch} (m^3)	$1 \cdot 10^{-5}$	10 mL
Diffusion coefficient of skin, $D_s (\frac{m^2}{s})$	$2.88689 \cdot 10^{-10}$	[4] *Molecular weight (MW) in Equation 5 was used as 2579 g/mol **The diffusion coefficient of the skin and the membrane were both assumed to be the same because they are both porous membranes.
Thickness of skin, x_s (m)	0.001	[6]
Volume of adhesive,	$100 \cdot 10^{-6}$	Cross - sectional area · thickness of adhesive (estimated)

$V_{adhesive} (m^3)$		to be 100 micrometers)
Volume of blood, $V_{blood} (m^3)$	0.005	Volume of blood ranges from 4.7 L to 5 L (according to Google)

It was not possible to find literature values for the diffusion of our peptide, BBI, through our patch's size controlling membrane and the skin. For this reason, it was assumed that the diffusion coefficient of the skin and size controlling membrane was $2.88689 \cdot 10^{-10} \frac{m^2}{s}$, as calculated from equation 5 [4]. Although this does not produce data that exactly represents the diffusion process of BBI in our system, it does reveal the general pattern of how it would diffuse.

PRODUCTION AND DEGRADATION RATE

The **production rate** is the amount of peptide (BBI) produced by the bacteria (*B. subtilis*) in a given period of time. In this model, it was assumed to be 1 mg/L [5].

The **degradation rate** is the amount of peptide lost in a given period of time through enzyme degradation in the liver and excretion through the kidneys. The equation to represent the degradation rate is determined to be:

$$degradation\ rate = k \cdot C_2 \quad (9)$$

Where:

k = decay constant (s^{-1})

To determine the decay constant, the exponential decay equation was used:

$$N(t) = N_0 e^{-kt} \quad (10)$$

Rearranging equation (10) for k :

$$k = \frac{\ln(\frac{N(t)}{N_0})}{-t} \quad (11)$$

Half-life conditions for the peptide were used in equation (11) to solve for k . The values used were the following:

Table 2: Values Used in Equation (11)

Parameter	Value	Where Value Is Obtained From
Time, t (s)	16200	The half-life of the peptide
$\left(\frac{\text{Concentration at time } t, N(t)}{\text{Initial concentration, } N_0}\right)$	0.5	At the half life, this ratio will always be 0.5 because it has half the amount of peptide from the original amount

After solving for k , it was found that its value was $4.2786863 \cdot 10^{-5} \text{ s}^{-1}$. Thus, equation (9) can be rewritten to be:

$$\text{degradation rate} = 4.2786863 \cdot 10^{-5} \text{ s}^{-1} \cdot C_2 \quad (12)$$

Now that the production and degradation rates were determined, these values, as well as table 1, were plugged into equations (4), (6) and (8). These equations were then solved through MATLAB by using the codes found in Appendix A. The following graphs for the concentration vs time for the different locations were determined.

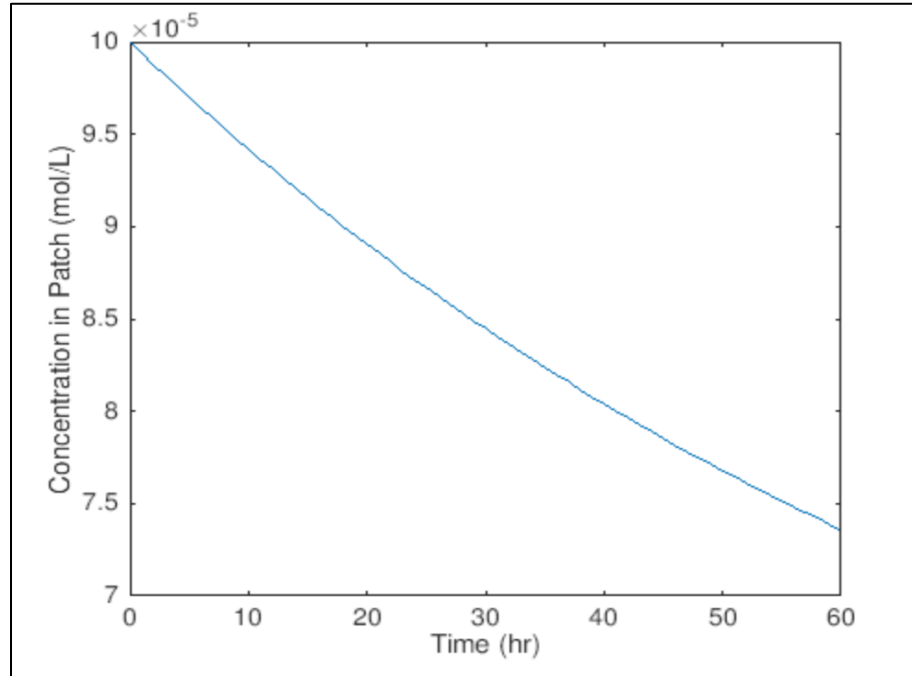


Figure 3: Concentration of BBI in the patch vs time

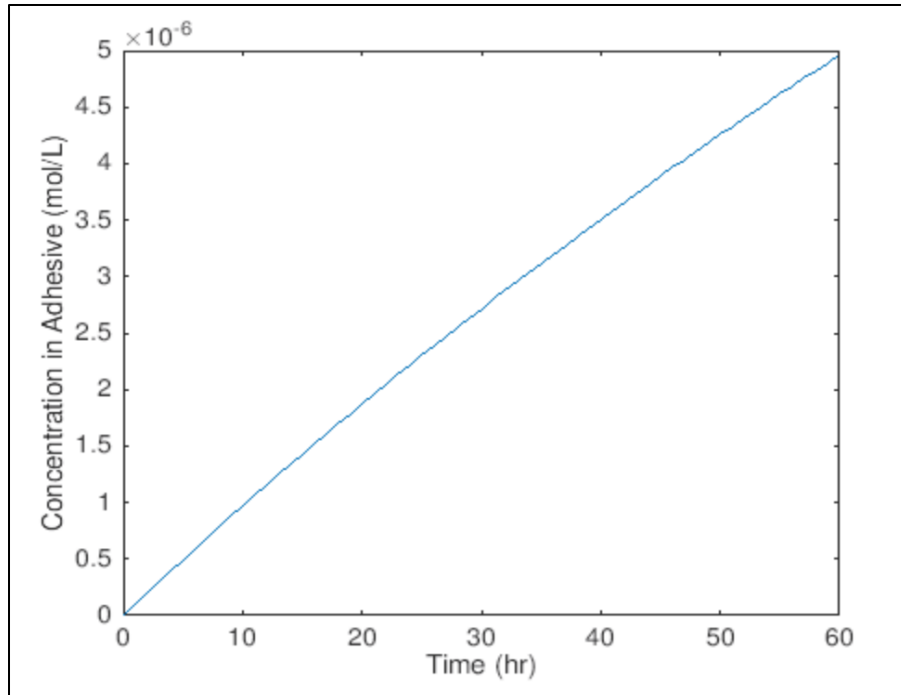


Figure 4: Concentration of BBI in the adhesive vs time

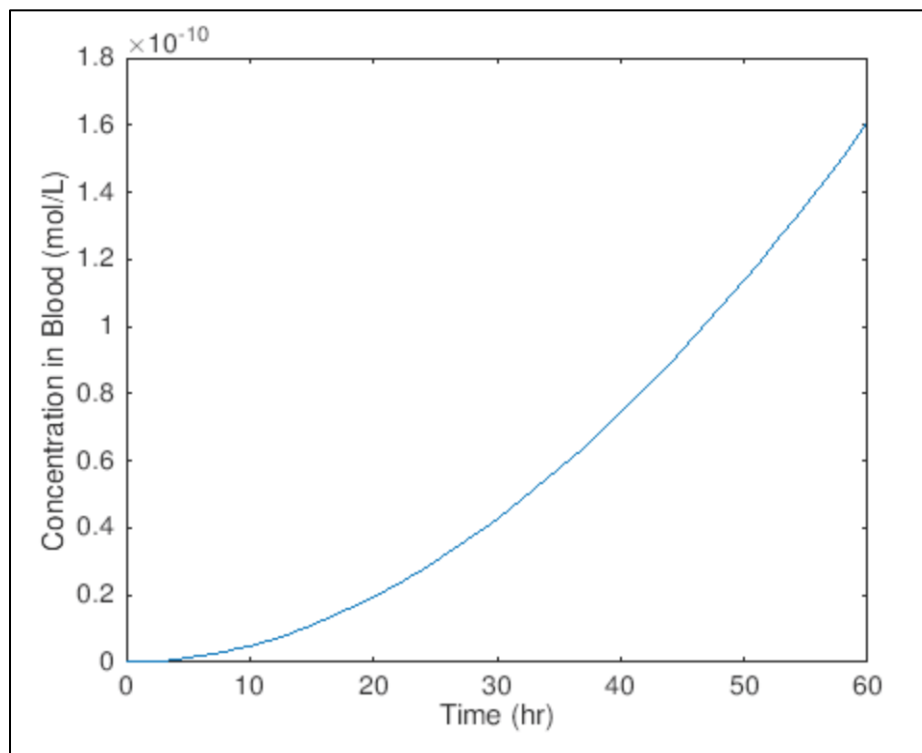


Figure 5: Concentration of BBI in the blood vs time

From these graphs, we were able to make a few conclusions to our initial problems. The peptide does not reach a constant concentration in the blood while the patch is on the user. (i.e.

in 60 hours); this was proved by Figure 3. The time was extended to see when the peptide concentration in the blood would reach steady state, and this was found to be an extremely large number (the peptide reached steady state concentration at a time much greater than 4 weeks, or 1 month). Figure 3 also determined that within 60 hours, the peptide does not reach 10 μM (the radioprotection minimum).

This suggests that in the future, the materials used in the patch needs to be reconsidered (e.g. a size-controlling membrane with a higher diffusion coefficient needs to be used), and other design considerations need to be investigated (e.g. finding a way to increase the lifetime of the bacteria creating the peptide so the peptide can be produced for longer periods of time). As seen from the figures produced from this model, this model can be used to determine:

- Whether the peptide reaches a steady concentration in the blood
- What this steady concentration is and how long it takes to reach the steady concentration
- Modifications can be made to the code to look at what the concentration over time is for other locations, such as in various layers of the skin
- Modifications can be made to the code to see what happens to the concentration in the blood over time after the patch has been removed. This is important to look at in the future, as it could help answer questions such as, exactly when the user should put on a new patch (i.e. when the concentration in the blood reaches zero again)

SUMMARY OF RESULTS

- The patch can be applied for 60 hours (about 2.5 days).
- The packets need to be popped every 12 hours.
- Figure 5 shows that BBI does not reach a constant concentration in the blood in 60 hours, and does not reach the radioprotective concentration of 10 μM . Hence, for the future, the design of the patch needs to be reconsidered (e.g. such as using materials that make it easier for the peptide to diffuse through).
- This model can be used as a tool when redesigning the patch in the future, because it can help determine things such as when the peptide reaches a steady concentration in the blood and how long it takes for the peptide concentration to go back to zero after the patch has been removed.

REFERENCES

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APPENDIX A

MATLAB CODES

MAIN CODE – diffcode.m

```
clear;
clc;

%Solving the system of ODE equations for the first 60 hours (when patch is on skin).
[time, concentrations] = ode45(@diff_mod, [0:3600:216000], [100*(10^(-6)),0,0])

time_hours = [0:1:60]

%Plotting concentration with patch only (60 hours total)
figure
plot(time_hours,concentrations(:,1))
xlabel('Time (hr)')
ylabel('Concentration in Patch (mol/L)')

figure
plot(time_hours,concentrations(:,2))
xlabel('Time (hr)')
ylabel('Concentration in Adhesive (mol/L)')

figure
plot(time_hours,concentrations(:,3))
xlabel('Time (hr)')
ylabel('Concentration in Blood (mol/L)')
```

FUNCTION – diff_mod.m

```
function [ret] = diff_mod(t,c)
ret = zeros(3,1);

x = c(1);
y = c(2);
z = c(3);

ret(1) = ((1.077075525*(10^(-7))) + ((2.784598622*(10^(-3)))*(y-x)))/(1000);
ret(2) = (((-2.784598622*(10^(-4)))*(y-x)) + ((1.4145761*(10^(-5)))*(z-y)))/(1000);
ret(3) = (((-2.8291522*(10^(-7)))*(z-y)) - (4.2786863*(10^(-5))*(z)))/(1000);
```